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Prokaryotic tubulin homologs
Prokaryotické homology tubulinu

Bachelor´s thesis

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Declaration

I declare that I wrote this thesis on my own and that I stated all used literature and other information sources. I did not use this work or its significant part to previously acquire any other academic title.

In Prague, 25.4.2018

Abstract

Cytoskeletal proteins form very important structures in eukaryotic cells. It was demonstrated in last years that cytoskeletal proteins are also an important part of prokaryotic cells. One of the cytoskeletal families is the tubulin family. The tubulins are known to form dynamic microtubules which are important for cell division and other processes in eukaryotic cells. Proteins homologous to tubulin were discovered in Prokaryotes, based on their similarity to the tubulin GTP-binding region. This thesis describes the structure and function of known tubulin homologs from bacteria and archaea and compares them to their eukaryotic counterplayers.

Abstrakt

Proteinu cytoskeletu tvoří velmi důležité struktury v eukaryotických buňkách. V posledních letech se ukazuje, že cytoskeleton je přítomný i v prokaryotických buňkách. Jednou z rodin cytoskeletárních proteinů je tubulinová rodina. Tubuliny tvoří dynamické mikrotubuly, které jsou důležité pro buněčné dělení a další buněčné pochody eukaryotických buněk. Proteiny homologní tubulinu byly také popsány v prokaryotech na základě sekvenční podobnosti GTP-vazebné oblasti. Tato bakalářská práce se zabývá popisem struktury a funkce známých bakteriálních a archealních tubulinových homologů a porovnává je k jejich eukaryotickým protějškům.

Keywords

Prokaryotic cytoskeleton, tubulin homologs, cytoskeleton, tubulin superfamily, FtsZ, BtubA/B, TubZ, CetZ, artubulin, eukaryotic evolution

Klíčová slova

Prokaryotický cytoskelet, tubulinové homology, cytoskeleton, tubulinové superrodina, FtsZ, BtubA/B, TubZ, CetZ, artubulin, evoluce eukaryot

List of Abreviations

FtsZ = filamentous temperature sensitive Z

BtubA/B = bacterial tubulin A/B

TubZ = tubulin/FtsZ

CetZ = cell-structure-related Euryarchaeota tubulin/FtsZ homologs

FtsZL1 = FtsZ-Like group homologs

GTP = guanosine triphosphate

MTOC = mikrotubule organizing center

MAP = mikrotubule asociated protein

Table of contents

1. Introduction 1

2. Tubulin homologs 3

2.1. α- and β-tubulin 3

2.2. FtsZ 6

2.3. BtubA/B 10

2.4. TubZ 15

2.5. CetZ 21

2.6. Other tubulin superfamily sequences in Archaea 23

3. Conclusion 27

4. References 28

1. Introduction

It has been 27 years since the first prokaryotic cytoskeletal protein was discovered. It was a bacterial homolog of tubulin – FtsZ protein, described by three laboratories at the same time (Bork, Sander and Valencia, 1992; de Boer, Crossley and Rothfield, 1992; Mukherjee, Dai and Lutkenhaus, 1993). Since then more homologs have been found in bacteria and archaea. Despite the overall low sequence homology, the proteins share signature amino-acid sequences in their active domains as well as structural and functional properties with their eukaryotic counterparts. Various groups of prokaryotic cytoskeletal proteins were described; homologues of tubulin (Vaughan *et al.*, 2004; Yutin and Koonin, 2012), actin (Toro-Nahuelpan *et al.*, 2016, Kruse and Gerdes, 2005) and also a homologue of intermediate filaments (Charbon, Cabeen and Jacobs-Wagner, 2009; Esue *et al.*, 2010). Moreover, proteins with cytoskeletal function and dynamics but with no defined homology to the eukaryotic cytoskeleton were described (Surovtsev, Campos and Jacobs-Wagner, 2016). The prokaryotic cytoskeletal proteins play a role in cell division (Bisson-Filho *et al.*, 2017), DNA segregation (Aylett *et al.*, 2010; Brzoska *et al.*, 2016) and often in cell shape determination (Esue *et al.*, 2010; Duggin *et al.*, 2015; Oswald *et al.*, 2016). Specific functions can be found, such as magnetosome chain assembly in magnetotactic bacteria (Toro-Nahuelpan *et al.*, 2016) and possible host protection by bacterial ectosymbionts of ciliate *Euplotidium* (Rosati *et al.*, 1999). The diversity of prokaryotic cytoskeletal proteins can be appreciated by inspecting Table 1.

A large number of prokaryotic cytoskeletal proteins is already known and it is highly possible that more will be discovered. The complete collection of prokaryotic cytoskeletal proteins would be too broad a subject for a bachelor thesis. Therefore I will focus on a group of tubulin prokaryotic homologs. The group is fairly divergent in structure and function and can demonstrate the variability of the prokaryotic cytoskeleton.

This thesis aims to describe the known tubulin prokaryotic homologs structure, dynamics, and function and to summarize the present opinion on their evolution mainly in regard to the emergence of the eukaryotic tubulin.

Protein	Eukaryotic homolog	Function	Distribution	References
FtsZ	tubulin	cell division	most Bacteria and Archaea	Vaughan *et al*., 2004; Lutkenhaus, 1993
BtuB/A	tubulin	unknown	Bacteria (*Prosthecobacter* spp.)	Deng *et al*., 2017
TubZ	tubulin	plasmid segregation	Bacteria (*Bacilli* plasmids)	Larsen *et al.*, 2007; Ni *et al.*, 2010
CetZ	tubulin	cell shape control	Euryarchaeota	Vaughan et al., 2004; Duggin *et al*., 2015
FtsZL1	tubulin	unknown, possible membrane remodelling	some Bacteria and Archaea	Makarova and Koonin, 2010
Artubulins	tubulin	unknown	Thaumarchaeota	Yutin and Koonin, 2012
MreB	actin	cell wall synthesis organization	most Bacteria	Oswald *et al.*, 2016, Figge *et al.*, 2004
ParM	actin	plasmid segregation	some bacterial plasmids, some Archaea	Bharat *et al*., 2015; Brzoska *et al*., 2016
MamK	actin	magnetosome chain assembly	magnetotactic Bacteria	Toro-Nahuelpan *et al*., 2016
Crenactin	actin	presumably cell division	some Crenarchaeota	Izoré *et al.*, 2014
CresC	intermediate filaments	cell shape	Bacteria (*Caulobacter* spp.)	Esue *et al.*, 2010
ESCRT III	eukaryotic ESCRT system	cell division	diverse Archaea, common in Crenarchaeota	Samson *et al*., 2008
ParA/B	no homology	DNA segregation	bacterial plasmids and chromosomes	Roberts *et al*., 2012; Surovtsev *et al.*, 2016
SegA	no homology	chromosome segregation	some Archaea	Kalliomaa-Sanford *et al.*, 2012
Bactofilins	no homology	flagellar hook establishment, mostly unknown	widespread in G- Bacteria	El Andari et al., 2015
CrvA	no homology	cell curvature	Bacteria (*Vibrio* spp.)	Bartlett *et al.*, 2017

Table 1: Prokaryotic cytoskeletal proteins

Representation of some prokaryotic cytoskeletal proteins. Proteins with no homology to eukaryotic cytoskeletan are called cytoskeletal due to their filamentous nature and/or cytoskeletal function in cells.

2. Tubulin homologs

The prokaryotic tubulin homologs share (with one exception described later - BtubA/B) an overall low sequence similarity with the eukaryotic tubulins. They are designated as homologous on the basis of the resemblance in some of the tubulin conserved regions. Subsequent protein studies (if realized) then often revealed alikeness in secondary structure and dynamic properties.

The eukaryotic tubulin group consists of six families of tubulin: α, β, γ, δ, ε, and ζ tubulins (Findeisen *et al.*, 2014). However, the prokaryotic homologs are mainly compared to α- and β-tubulins as they have been known for the longest time and they are well understood. Therefore, a short overview of eukaryotic α- and β-tubulin is provided.

2.1. α- and β-tubulin

The α- and β-tubulin form a heterodimer and then polymerize into hollow tubes mostly consisting of 13 protofilaments, called microtubules (Sui and Downing, 2010; Chaaban and Brouhard, 2017). The α- and β-tubulins have approximately the same molecular weight of 53 – 55 kDa and they share a 40 % sequence similarity (Wolf, Nogales and Downing, 1998). The 3D structure of the two proteins is also very similar. Both proteins have an N-terminal GTP-binding domain containing a typical fold for nucleotide-binding proteins (the Rossmann fold). The crystal structure of the tubulin dimer can be observed in Figure 1. The parallel β-sheet from strands S1 to S6 is enclosed by α-helices H1 to H6. The N-terminal domain is separated from the C-terminal domain by the intermediate H7 α-helix. The C-terminal domain consists of two parts – one with α-helices H8 and H9 and a mixed β-sheet from strands S7 to S10 and the second from α-helices H11 and H12. These two C-terminal α-helices cover the subunits from the outer side of the formed microtubule and are therefore well approachable for proteins interacting with the microtubule (Wolf, Nogales and Downing, 1998; Löwe *et al.*, 2001). The C-terminal domain is the most variable among the tubulin families and also among the tubulin homologs (Vaughan *et al.*, 2004; Findeisen *et al.*, 2014).

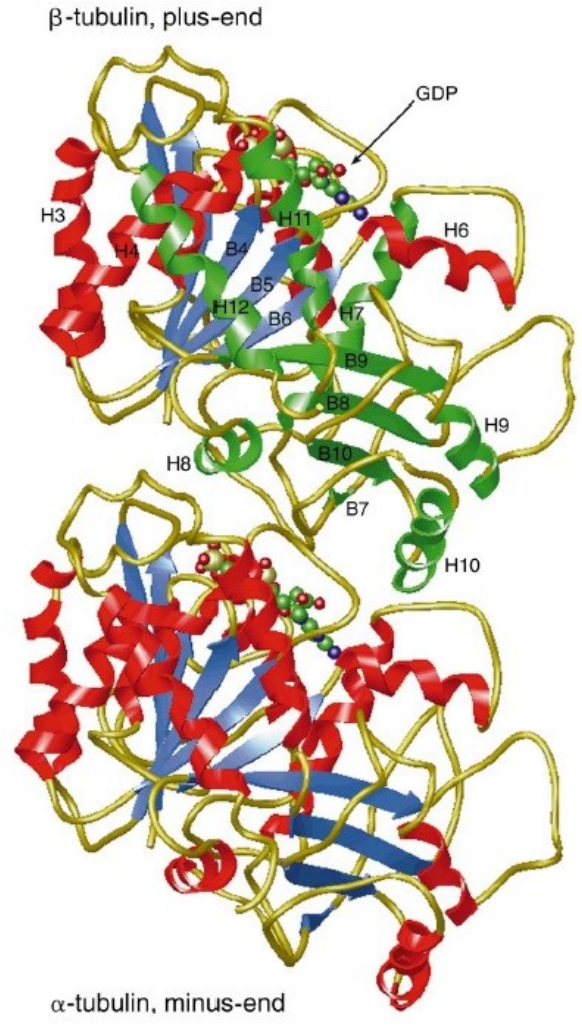

Figure 1: The crystal structure of tubulin dimer as crystallized in presence of stabilizing drug taxol.
The β-tubulin C-terminal domain is in green. The rest is colored in blue for β-strands, in red for α-helices and in yellow for loops.
The β-tubulin is on top and it binds a GDP molecule. The α-tubulin is on the bottom and it binds a GTP molecule (adapted from Erickson, 1998)

Both the α- and the β- subunit bind a GTP molecule. However, only the GTP on the β-tubulin is exchangeable and can be hydrolyzed to GDP. The GTPase activity of the β-tubulin is activated only after the dimerization (Nogales *et al.*, 1999; Sui and Downing, 2010). The GTP on the α-subunit is permanently bound and never hydrolyzed. The GTP-binding site of the α-subunit is enclosed in the longitudinal contact site between the two subunits, this position prevents the exchange of the GTP (Wolf, Nogales and Downing, 1998; Löwe *et al.*, 2001).

The tubulin dimer assembles with other dimers to form short polymers. These can proceed to the phase of elongation when microtubules are formed by polarized growth (Detrich *et al.*, 1985; Caudron *et al.*, 2002). In the polarized growth phase, the GTP-bound dimers are added to the plus end of the nascent microtubules where the β-subunits are exposed. The dimers with GDP-bound β-subunit dissociate on the minus end as they hydrolyze the GTP during their passage through the length of the microtubule. This polarized growth is called treadmilling (Tran, Walker and Salmon, 1997; Alushin *et al.*, 2014). The microtubule polymerization is usually supported by different proteins in cells such as γ tubulin which form MTOCs (microtubule organizing centers). These can also stabilize the minus ends of the microtubules (Kollman *et al.*, 2010). The GTP-bound dimers on the plus end form a cap preventing the microtubules from another event - a catastrophe. When the GTP-cap is hydrolyzed the plus

end is no longer protected from the depolymerization and the microtubule rapidly depolymerizes. The protofilaments may form ring-like structures as they unwind from the microtubule. These catastrophes are common in the cells and the dynamic instability of microtubules is important for their function in cells. Schematic representation of the two dynamic properties is depicted in Figure 2 (Mitchison and Kirschner, 1984; Hyman and Mitchison, 1990; Caplow and Shanks, 1996).

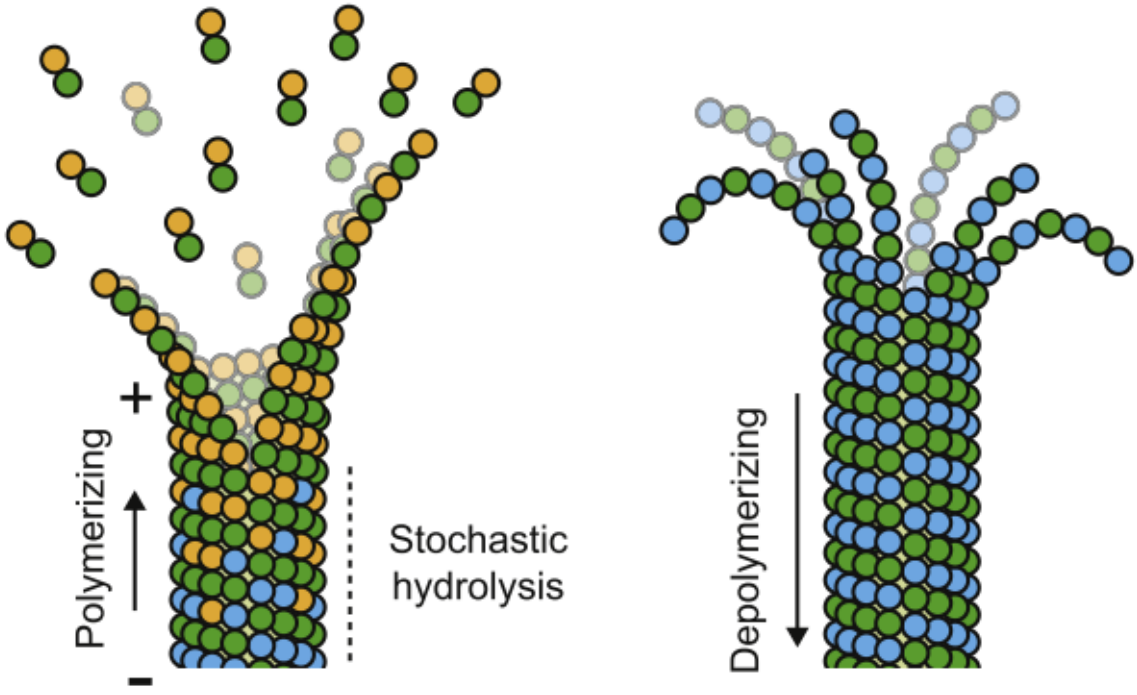

Figure 2: The treadmilling and dynamic instability of microtubules. The green spheres represent α-tubulin with permanently bound GTP, the orange spheres represent GTP-bound β-tubulin and the blue spheres GDP-bound β-tubulin. The microtubule on the left undergoes polarized growth; treadmilling. The microtubule on the left lost its GTP-cap and it is rapidly depolymerizing (adapted from Alushin *et al.*, 2014)

Microtubules interact with many other proteins which can change their dynamics through posttranslational modifications (Xu *et al.*, 2017), connect them to other cytoskeletal networks (Lee and Kolodziej, 2002) or serve as a motor for intracellular transport (Gigant *et al.*, 2013). These proteins often interact with the C-terminal region of the subunits (Cross *et al.*, 1991). Microtubules play diverse roles in the functioning of eukaryotic cells including DNA partitioning, cell division, intracellular transport and ciliary and flagellar motility (Yang, Ganguly and Cabral, 2010; Linck *et al.*, 2014).

2.1. FtsZ

FtsZ (filamentous temperature sensitive Z) protein was the first cytoskeletal protein discovered in prokaryotes. Immunoelectron microscopy showed the FtsZ protein dispersed in the cytoplasm of growing cells but forming a ring in the midpoint of dividing cells. It was proposed that FtsZ is responsible for cytokinesis. The ring was henceforth denoted as a Z-ring (Bi and Lutkenhaus, 1991).

FtsZ has been found in most bacteria so far, only members of Chlamydiaceae family and *Ureaplasma urealyticum* lack FtsZ. It is possible that these organisms lost FtsZ due to their parasitic style of life (Vaughan *et al.*, 2004). FtsZ is also shared by most of archaea, FtsZ is completely missing only from Crenarchaeota (Baumann and Jackson, 1996; Moriscot *et al.*, 2011). Many Archaea have two FtsZ proteins from separate families - FtsZ1 and FtsZ2, some have only one of them (Vaughan *et al.*, 2004). The archaeal FtsZ is less studied than the bacterial FtsZ yet the fundamental characteristics seem to be the same.
FtsZ has a low sequence similarity with eukaryotic tubulin; around 20% (BLAST search; Stephen F. Altschul *et al.*, 1997) Nevertheless, the structural homology between FtsZ and tubulin is high as can be seen in Figure 3. Both proteins form two domains connected by an intermediate α-helix. N-terminal domains share a classic nucleotide-binding fold of β-sheets surrounded by α-helices. They share the typical tubulin GTP-binding motif consisting of several loops and helices which contact the GTP molecule. The amino acid sequences of these parts are highly conserved (Löwe and Amos, 1998; Findeisen *et al.*, 2014). FtsZ from some organisms have an extra N-terminal H0 α-helix protruding from the N-terminal domain. This helix is flexible and it forms one of the sites of contact between subunits in FtsZ protofilament (Oliva, Cordell and Löwe, 2004). The proteins differ in C-terminal domains. Tubulin C-terminal domain consists of four β-strands and five surrounding α-helices and the very C-terminal part constituting of two helices H11 and H12. It serves as a MAP (=microtubule associated proteins) binding site (Wolf, Nogales and Downing, 1998; Kong *et al.*, 2015; Xu *et al.*, 2017). On the other hand, FtsZ C-terminal domain is much smaller. It contains four β-strands (three parallel, one antiparallel) supported by two α-helices (Löwe and Amos, 1998). The domain also serves as an interaction site for proteins FtsA and ZipA. Both FtsA and ZipA are membrane-tethering proteins connecting FtsZ to the cytoplasmic membrane of the cell (Ma and Margolin, 1999; Rueda, Vicente and Mingorance, 2003; Pichoff and Lutkenhaus, 2005).

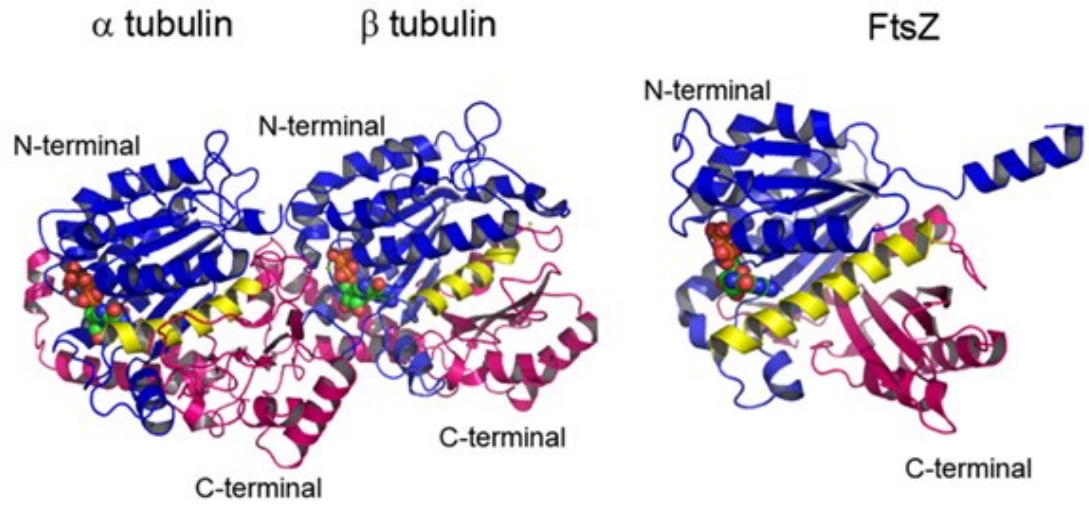

Figure 3: Structural similarities between FtsZ and tubulin dimer
Ribbon model depicting crystal structure of αβ tubulin dimer and FtsZ monomer. The C-terminal domain is in pink, central α-helix in yellow and N-terminal domain in blue. The protruding N-terminal α-helix can be observed in FtsZ structure and the differences between structure of C-terminal domains is obvious. The α tubulin binds GTP, the β tubulin binds GDP as does FtsZ (ball and stick model) (adapted from Battaje and Panda, 2017)

The FtsZ was observed to assemble into straight protofilaments, curved protofilaments forming minirings and 2D sheets of protofilaments *in vitro*. The sheets have similar lattice to the tubulin wall lattice and the minirings are similar to the rings formed by protofilaments upon microtubule depolymerization (Erickson *et al.*, 1996; Housman *et al.*, 2016). However, FtsZ was not observed to form any sheets or microtubule-like structures *in vivo*.
The physiological function of FtsZ in prokaryotic cells is connected to the formation of the cytokinetic Z-ring. *Escherichia coli* with mutant FtsZ fails to divide and forms abnormally long cells. Overexpression of FtsZ leads to the defective division on poles of the cells and budding of small nonnuclear bodies (Lutkenhaus, 1993; Szwedziak *et al.*, 2014).

There is an ongoing debate about the precise role of FtsZ in cytokinesis whether FtsZ provides the main constriction force or if it is only a scaffold protein. The proposed models are often based on *in vitro* experiments with liposomes, small vesicles from lipid membrane. The liposomes can be filled with the desired proteins without unknown interference.

A cryoelectron microscopy and tomography of liposomes containing FtsZ showed one long protofilament forming a spiral. It was demonstrated, that this artificial Z-ring can constrict liposomes and even finish membrane scission in presence of FtsA protein which tether FtsZ to the membrane. The proposed mechanism included sliding of protofilament along itself resulting in contraction and reduction in diameter of Z-ring (Szwedziak *et al.*, 2014). However, no motor protein or another way how to generate necessary force has been discovered.

It was shown that FtsZ protofilaments gain curved morphology upon GTP hydrolyzation. In another experiment with liposomes, FtsZ with added membrane targeting sequence was able to provide constriction of the membrane but was not able to finish scission (Osawa, Anderson and Erickson, 2008). The scission was completed in presence of FtsA. It was demonstrated that FtsA is important not only for protofilaments assembly on the membrane but also for FtsZ turnover (Loose and Mitchison, 2014).

In vivo, Z-ring morphology was different in some observed species. Helical nature of Z-ring was confirmed but a Z-ring consisting of overlapping short protofilaments was often observed ,rather then one long protofilament (Fu *et al.*, 2010; Anderson, Gueiros-Filho and Erickson, 2004). The FtsZ-ensured constriction was limited only to liposomes, with no cell wall or high turgor pressure. The force developed by simple protofilament curvation would not, presumably, be enough to overcome cell turgor pressure and to bend the plasma membrane. The alternative hypothesis suggests that the cell wall remodeling is needed to provide enough force for membrane bending and constriction and that the remodeling is a primary source of constriction force (Lan, Wolgemuth and Sun, 2007). FtsZ works as a scaffold protein for the division proteins complex and cell wall remodeling proteins (Typas *et al.*, 2017). Consistent with this is a study using correlative cryo-microscopy and cryo-electron microscopy of *E. coli* cells. The authors compared the colocalization of membrane invaginations with FtsZ and with peptidoglycan synthesizing enzymes during early stages of the cell division. They found out that the FtsZ is not sufficient for the membrane constriction *in vivo* as the invagination only occured in absence of the peptidoglycan synthesizing enzymes (Daley, Skoglund and Söderström, 2016). A recent study showed that FtsZ protofilament treadmills and that this treadmilling is very important for septum formation. Protofilament grows on one end while simultaneously depolymerizes on the opposite end. Cell wall remodeling complex trails the growing end which results in the uniform growth of septum around the division site. This would also explain why cells do not divide properly when the GTPase activity of FtsZ is inhibited since treadmilling is based on GTP hydrolysis (Yang *et al.*, 2017).

Interestingly enough, FtsZ is preserved in some semiautonomous organelles of the eukaryotic cells. It is a key player in chloroplast division, from algae to higher plants. Furthermore, chloroplast FtsZ is related to FtsZ of Cyanobacteria, which are the closest relatives to the bacterial ancestors of chloroplasts (Terbush *et al.*, 2017; Gilson and Beech, 2001). Plastid division complex constitutes of several protein rings, FtsZ forms a ring on the inner side of the inner membrane probably functioning in a scission of the inner membrane. The outer membrane is constricted by a ring formed by dynamin-related GTPase on the cytoplasmic side of the chloroplast (Miyagishima *et al.*, 2003). Proteins of dynamin superfamily are involved in membrane remodeling of eukaryotic cells. Dynamin itself is known to drive scission of vesicles (Danino, Moon and Hinshaw, 2004).

Mitochondrial FtsZ is absent from higher eukaryotes such as animals or plants, it was identified only in some algae and protists. Mitochondrial FtsZ of these organisms is related to FtsZ from α-proteobacteria (bacterial ancestors of mitochondria) and again forms a ring attached to the inner membrane.

Dynamin-related GTPases are also needed for mitochondrial fission. They again constrict mitochondria from the cytoplasmic side. The role of dynamin-related GTPases is widespread in animal and plant mitochondria in contrast to the role of FtsZ. (Kageyama, Zhang and Sesaki, 2011; Nishida *et al.*, 2003).

FtsZ protein is similar to tubulin but it also has unique features. FtsZ could be inhibited by specific antibiotics (with no influence on tubulins) and therefore represents a selective bacterial target. A wide range of small compounds and peptides have been tested for their effects on FtsZ (Araujo-Bazan *et al.*, 2016). FtsZ can be important for an insight into organel evolution as FtsZ is needed for division of chloroplasts and is present in mitochondria of "lower" eukaryotes. It would be interesting to investigate why FtsZ was lost from mitochondria of higher eukaryotes but it was preserved in chloroplasts.

2.2. BtubA/B

BtubA and BtubB (bacterial tubulins A and B) are homologs of α- and β-tubulin found in genus *Prosthecobacter* (phylum Verrucomicrobia). They show high sequence identity with eukaryotic α- and β-tubulins (~35%). BtubA and BtubB genes form an operon together with Bklc (bacterial kinesin light chain). Bklc was proposed to be called "BtubC" as will be discussed later (Jenkins et al., 2002; Deng et al., 2017). BtubA/B also remarkably resembles tubulin in structure and dynamics.

BtubA/B share the secondary structure with tubulins with only slight differences. The N-terminal nucleotide-binding domain is separated from the intermediate domain by an H7 helix. The intermediate domain contains T7 loop for GTP hydrolysis activation of adjacent subunits during protofilament assembly. In contrast to tubulins, both BtubA and BtubB form T7 loop. The C-terminal domain is also very similar to tubulins. It is likely that the C-terminal domain is also a binding site for Bklc, as this is the place where MAPs interact with tubulins and the Bklc protein is homologous to kinesins light chains of various eukaryotic kinesins (Cross *et al.*, 1991; Akendengue *et al.*, 2017). Motor protein kinesin is bound to vesicles through its light chain. Bklc was also shown to bind BtubB to the membrane but no motor function was observed (Schlieper *et al.*, 2005; Akendengue *et al.*, 2017; Deng *et al.*, 2017). Both monomers hydrolyze GTP and therefore bind GDP at some point, as opposed to tubulin, where the α subunit binds nonexchangeable GTP (Wolf, Nogales and Downing, 1998; Schlieper *et al.*, 2005; Deng *et al.*, 2017). The structural comparison can be seen in Figure 4.

The monomers of BtubA/B bind each other to form a heterodimer in presence of GTP. Only heterodimers can further assemble to form protofilaments. Monomers with mutations in sites of longitudinal contact between monomers and therefore defective dimer binding can even induce depolymerization of already formed protofilaments (Sontag, Sage and Erickson, 2009).

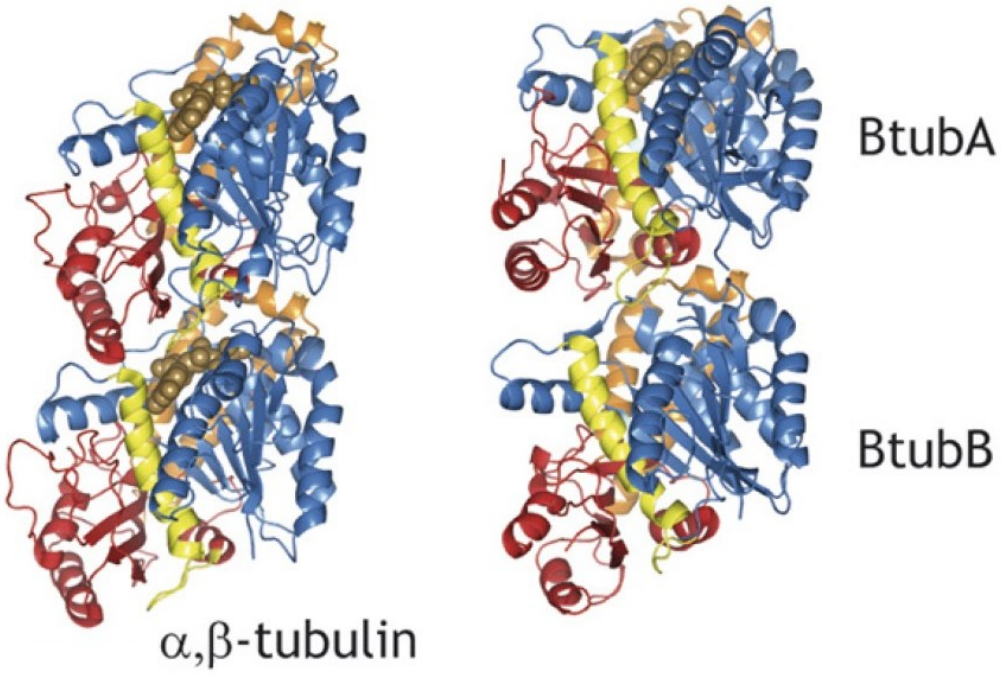

Figure 4: The comparison of the tubulin and the BtubAB dimer
The N-terminal domain is in blue the intermediate helix in yellow. The C-terminal domain is separated into more parts in this picture; the GTPase-activating intermediate domain in red and the very C-terminal part in gold (adapted from Martin-Galiano *et al.*, 2011).

The most interesting feature of Btubs is their ability to form microtubule-like structures. These were observed in a study performing electron microscopy of different Prosthecobacter species. The tubules were observed in all BtubA/B-positive species and also in recombinant Escherichia coli with inserted btubs genes but the number of tubules was different in different species and growth conditions. The tubules traversed through the whole length of the cells either separately or in bundles. It was proposed that the tubules constitute of five protofilaments. The tubules did not have a twist and they were proposed to have a microtubule-like lattice; the B-lattice where the A and B subunits do not alternate and a seam where the A and B subunits adjoint (as in Figure 5) (Pilhofer *et al.*, 2011). However, four-protofilament tubules were observed in experiments with *in vitro* polymerized tubules. The structure was resolved with 3.6 Å resolution. The authors used Bklc protein to distinguish BtubA and BtubB from each other as Bklc binds only to BtubB (Deng *et al.*, 2017). The Bklc was proposed to be called BtubC for clarity as it forms tubules together with BtubA and BtubB (Deng *et al.*, 2017).

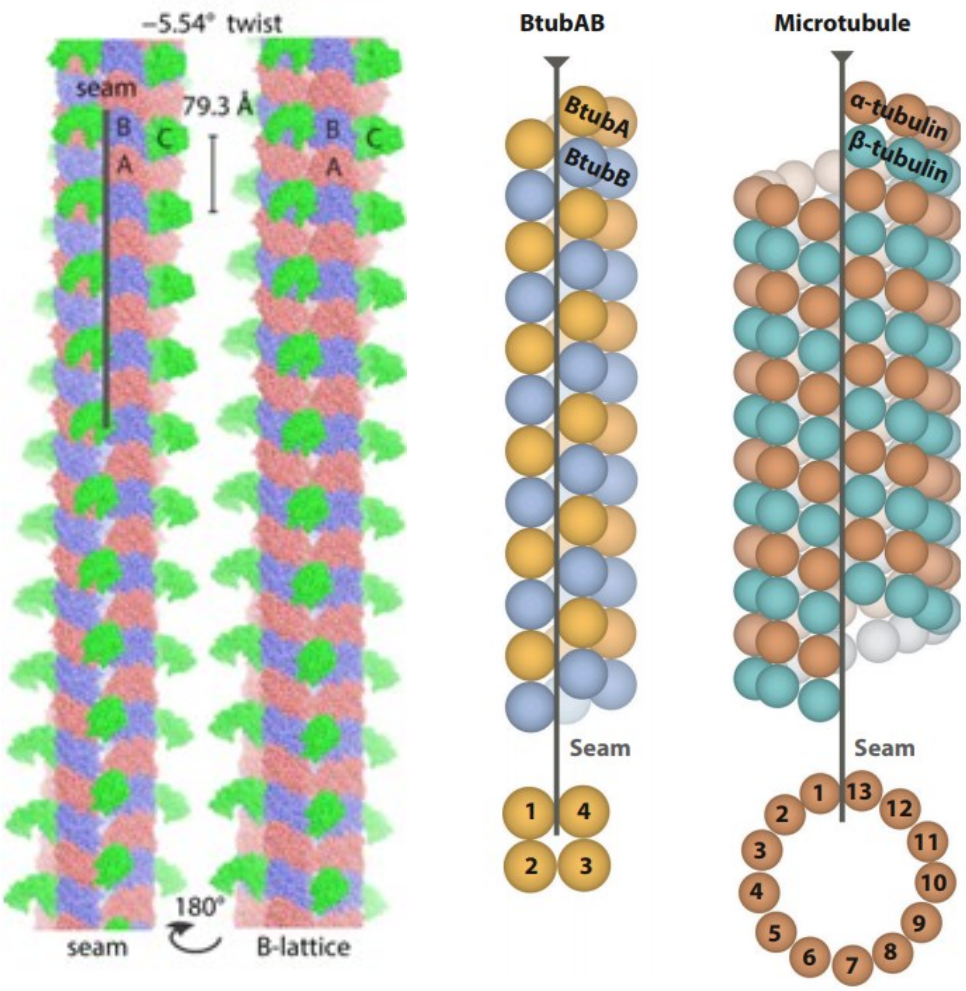

Figure 5: Structure of BtubABC minimicrotubules and their comparison to eukaryotic microtubules.
The three color model of BtubABC minimicrotubules is on the left. Minimicrotubules with a slight helical twist consists of A subunits in pink, B subunits in violet and BtubC in green. BtubC subunits bind to BtuB subunits. On the right is a comparison of microtubules and minimicrotubules. The minimicrotubules consist of four protofilaments, microtubules of 13 protofilaments. Each of the structures has a B lattice (A-A an B-B) and an A lattice or a seam (A-B) (adapted from Deng *et al.*, 2017).

The resolved structure using BtubC also showed that the "minimicrotubules" have a microtubule-like lattice with a seam (see Figure 5). The protofilaments are ~90° rotated against each other and the contact between them is mediated by a loop homologous to M-loop in tubulins. However, the M-loop is ordered only in BtubA, it is collapsed in BtubB in contrast to tubulins where both α- and β-subunits have an ordered M-loop (Wolf, Nogales and Downing, 1998; Deng *et al.*, 2017).

The models of five and four protofilaments can differ due to different polymerization environment (*in vivo* versus *in vitro*) or due to a lower resolution obtained by older methods in the earlier study.

It is intriguing that observed BtubAB minimicrotubules showed two of tubulin dynamic properties – treadmilling and dynamic instability. The latter was not observed in FtsZ protofilaments. The minimicrotubules have a plus end, where subunits are added and a minus end, where they depolymerize. This movement is connected with GTP hydrolyzation as subunits with GTP are bound and GDP subunits depolymerize. Furthermore, catastrophes occur when the protective GTP cap on the plus end is hydrolyzed and protofilaments loosen contact with each other. The catastrophe rate was very high when only BtubA and BtubB were present but the rate declined significantly in presence of BtubC. Also, the rescue events (event when the rapid depolymerization stops and the tubule starts growing again) were more abundant in presence of BtubC. Hence it can be stated that BtubC stabilizes minimicrotubules, at least in *in vitro* conditions (Deng *et al.*, 2017).

Various features of BtubA and BtubB speak for their acquisition by a horizontal gene transfer. The btub genes are found only in a small group within Verrucomicrobia and the genomes of this group are otherwise normal bacterial genomes. The similarities between tubulins and BtubA/B are so high that they must be homologous and it is unlikely that these specific proteins would appear only in a small group of bacteria. However, Btubs are capable of folding without chaperones in contrast to tubulins (Schlieper *et al.*, 2005). We can consider two alternative scenarios about the Btubs transfer. It might have happened by tubulin predecessor transfer into a Prosthecobacter ancestor from an older Eukaryote. This tubulin predecessor was able to assemble without chaperones and it formed thinner microtubules. Alternatively, a more modern tubulin could have been transferred into a Prosthecobacter ancestor but was then altered in such a way so it could assemble without chaperones and had a more suitable size for a bacterial cell. However, the problem with the latter scenario is a lack of function of the tubulin in the bacterial cell before it was able to fold without chaperones (Schlieper *et al.*, 2005; Pilhofer *et al.*, 2011).
Together with btuba/b genes *Prosthecobacter* also contains FtsZ gene (Pilhofer *et al.*, 2007). It is likely that FtsZ serves as a cell division protein in *Prosthecobacter*. The function of BtubA/B is yet to be discovered.

Lastly, Verrucomicrobia phylum also includes a special group of bacterial symbionts - epixenosomes. Epixenosomes are episymbionts of ciliates of the genus *Euplotidium* and they

can protect their host from predators by a special extrusive apparatus (Rosati *et al.*, 1999) Electron microscopy showed microtubule-like structures forming a basket of bundled tubules next to the extrusive apparatus. The functioning of extrusive apparatus is dependent on the tubules (Petroni *et al.*, 2000). It was also demonstrated that these tubules are sensitive to the microtubule drug nocodazole as well as low temperatures. The tubules can even be labeled by tubulin antibodies (Rosati, Lenzi and Franco, 1993). Although we can not be sure whether the tubules are formed by tubulin or how precisely did the symbionts acquire them, it is likely that they were obtained by a horizontal gene transfer. They may have gained them from their eukaryotic host. It would be interesting to check if these structures are related to BtubA/B minimicrotubules from Prosthecobacter. It is necessary to obtain epixenosomes genome and find out more about their phylogenetic assignment and protein nature of their tubules to answer some of these questions.

2.3. TubZ

The TubZ protein is a member of tubulin/FtsZ family related to both of these proteins. The similarities are implicated by TubZ´s name (Tubulin/FtsZ). It is one of the previously described FtsZ-like proteins (Vaughan *et al.*, 2004). The tubZ gene is present in various plasmids of bacteria from Bacillus genus (Larsen *et al.*, 2007). The TubZ sequence is closer to tubulin in some regions and to FtsZ in other. The overall sequence of the T4 loop is more similar to FtsZ. On the other hand, the T6 binding loop sequence is closer to tubulin. However, the overall sequence similarity to tubulin and FtsZ is only ~15% (Aylett *et al.*, 2010). Interestingly, the TubZ shares only part of conserved GTPase motif: GxxNxDxxD/E. The tubZ and tubZ-like genes contain the DxxD/E part but they differ in the first part of the motif (Larsen *et al.*, 2007). Similar mutations in FtsZ GTPase domain lead to severe dysfunction of FtsZ as it is unable to hydrolyze GTP and polymerize (Mukherjee, Saez and Lutkenhaus, 2001). However, TubZ has a functional GTPase domain.

The crystal structure of TubZ has, once again, the tubulin-like fold. The N-terminal domain contains a Rossmann fold of β-strands surrounded by α-helices binding GTP.

The C-terminal domain consists of four β-strands. Both terminal domains are connected by a long interdomain helix. TubZ has two more specific helices – H0 at the N-terminus and H11 at the C-terminus. H0 helix can also be found in FtsZ structure but the position of H0 differs in both proteins. H0 helix in FtsZ is protruding out of the compact structure whereas TubZ helix have multiple interactions with C-terminal domain and it is secured in its position (Ni *et al.*, 2010). On the other hand, the H11 helix is flexible and this flexibility is necessary for the filament assembly of TubZ. The H11 helix changes its positioning during assembly and GTP hydrolysis and enables the interesting TubZ protofilament changes, discussed below (Aylett *et al.*, 2010; Ni *et al.*, 2010). The crystal structures of TubZ and tubulin dimer are compared in Figure 6.

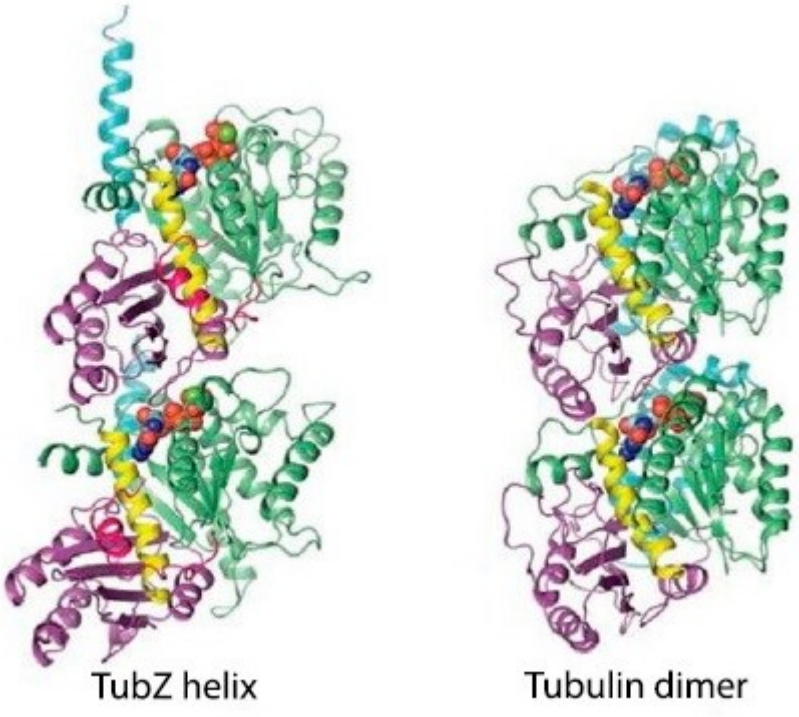

Figure 6: Crystal structure of TubZ dimer in comparison to tubulin dimer
The TubZ dimer is a part of the two-stranded filament which was polymerized in presence of GTP-γS – a slowly hydrolyzable GTP analog which stabilizes filaments. The N-terminal part is in light green, the intermediate helix is in blue, the C-terminal part in violet. The H0 helix in TubZ structure is in bright pink, it is enclosed in the C-terminal domain. The helix H11 is in cyan, it lies on the top of the N-terminal domain in tubulin dimer but it is directed upwards in TubZ (adapted from Aylett *et al.*, 2010).

TubZ protofilaments have an unusual right-handed twist. Protofilaments crystalized with GTP turn 360° over 14 subunits, GDP-bound protofilaments over 12 subunits. Neither tubulin or FtsZ protofilaments have a twist (Aylett *et al.*, 2010).
The protofilaments then assemble to form a two-stranded filament in presence of GTP. However, this two-stranded filament is only an intermediate as upon GTP hydrolyzation the filaments assemble further and form four-stranded filaments. The two- and four-stranded filaments copy the twist differences between GTP and GDP-bound protofilaments as stated earlier. Two stranded-filaments with most subunits bound to GTP have lower twist than the four-stranded filaments containing mostly GDP (Montabana and Agard, 2014).
The four-stranded version of the filament is more abundant and stable in the population of filaments formed in an environment with excess GTP even though the crystal structure of four-stranded filaments revealed looser contacts between subunits (Montabana and Agard, 2014). The two filaments are compared in Figure 7.

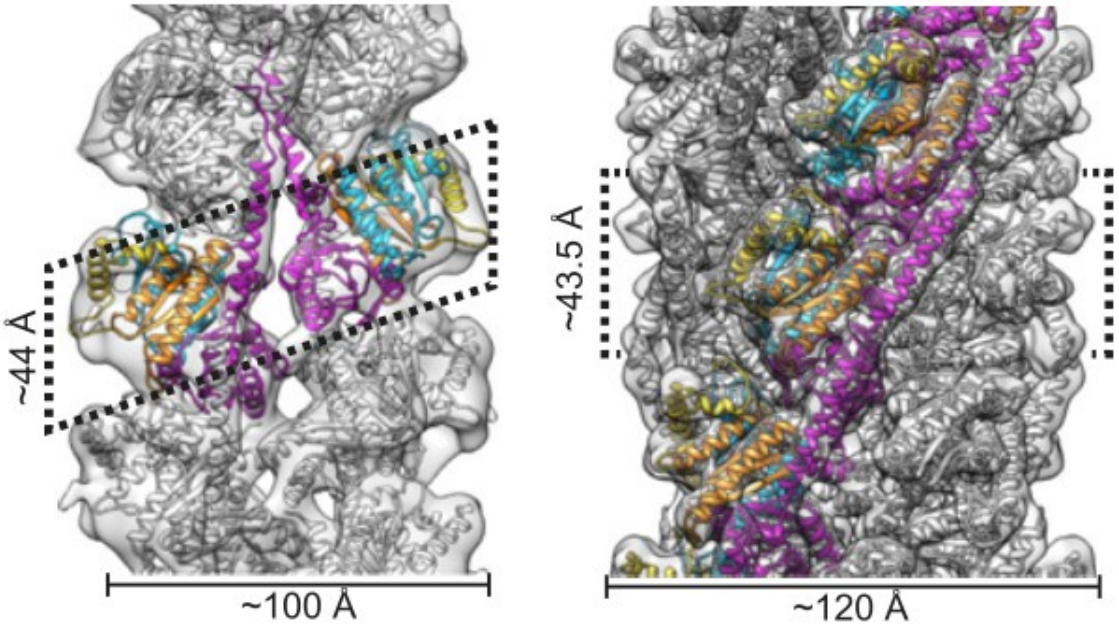

Figure 7: The two- and four-stranded TubZ filaments
Cryo-EM reconstruction of the two types of TubZ filaments. The left structure represents the two-stranded filament polymerized with GTP-γS. The right structure represents the four-stranded filament polymerized in presence of GTP which was hydrolyzed. The four-stranded filament is denser and has a lower twist. The N-terminal domain is in magenta, the H11 helix protrudes from the N-terminal domain (adapted from Montabana and Agard, 2014).

The reason for the higher stability of four-stranded filaments may be the presence of GTP cap which stabilizes the end of the filaments by a similar mechanism as in microtubules. This hypothesis is supported by *in vitro* experiments. Despite the filaments being almost 100% GDP bound they remain in the solution in presence of GTP. After the GTP pool exhaustion, the filaments depolymerize. However, in presence of slowly hydrolyzable GTP analog the filaments remain stable. This behavior is similar to the tubulin (Chen and Erickson, 2008).

The TubZ protein dynamics was characterized using *Bacillus thuringiensis* which contains tubZ gene on the pBtoxis plasmid. The pBtoxis is a low-copy virulence plasmid of *Bacillus thuringiensis* carrying genes for insecticidal toxins (Aronson, 1993).
TubZ-GFP formed dynamic filaments in *B. thuringiensis*. The polymers were long, sometimes spread through the cell from pole to pole. They showed directional movement around the cell in circular pathways. Filaments did not change their direction of movement. It was demonstrated by FRAP that the movement is powered by treadmilling. A small bleached area from plus end moved toward the minus end of the observed filament where it disappeared. There was no recovery of the bleached area fluorescence which would suggest

lateral subunit exchange. The rate of the movement was 1.5 um/min. No dynamic instability was observed. Nevertheless, the filaments completely depolymerized in starving cells incubated on glass slides with no nutrition. The disassembly rate was fast: 2.25 um/min and the filaments depolymerized from both ends (Larsen *et al.*, 2007). These observations support the model of TubZ dynamics as follows; the GTP cap on the plus end of filaments prevents rapid depolymerization from plus end but the GDP minus-end depolymerizes continually. These two phenomena result in treadmilling growth and movement of the filament. The filaments probably do not undergo catastrophes *in vivo.*

The physiological function of TubZ is in plasmid partitioning. The pBtoxis is a low copy plasmid and as such cannot rely on a simple diffusion for its transfer to daughter cells. The TubZ forms a partitioning complex together with the protein TubR and the centromeric DNA sequence tubC. The TubR protein binds to TubZ last 14 amino acids on the C-terminus. TubR is also a DNA binding protein and binds to the tubC sequence of the pBtoxis plasmid (Ni *et al.*, 2010). The tubC sequence consists of several direct repeats. The TubR binds to tubC cooperatively and the two parts together form the TubRC complex. The TubRC complex associates with the TubZ filaments and together they form the TubZRC complex. The TubRC complex was demonstrated to track the minus end of the growing TubZ filaments but never the plus end. TubRC also slowed the depolymerization rate from the minus end. Notably, the TubRC complex also promotes seeding of the filaments and this effect leads to an assembly of filaments at concentrations that are lower than the critical concentration of solely TubZ (Fink and Löwe, 2015).

How does the TubZRC complex partition the plasmids? It is possible that TubRC complex serves as a nucleation site for the TubZ filament and tracks its minus end as the filament treadmills around the cell. Only one filament per cell was observed to circle the cell, not multiple filaments which would be anticipated if the TubZ concentration was above the critical level in the cell. It is plausible that the TubRC complex unbinds the TubZ filament in place of its curving on the cell pole. The curving of the filament might induce a type of conformational change in the TubZ or TubRC. Nonetheless, it remains to be elucidated how the two copies of the plasmid are separated to the opposite poles. Perhaps the TubZ minus end can bind only one TubRC complex at a time and therefore always carry only one of the plasmid copies. The plasmid copy would then disengage at one of the poles and the TubZ filament would recruit the second copy during its passing through the middle of the cell. This second copy would then unbind the TubZ on the opposite pole from the first copy. More

molecules may participate in the whole mechanism and these may control the process through a mechanism that remains unknown to date. A model of TubZRC plasmid partitioning is depicted in Figure 8.

In any case, the TubZRC complex is very interesting because of its resemblance to the eukaryotic kinetochore. This tubulin-like system differs significantly from other plasmid partitioning systems using actin homologs ParA and ParM. In these systems the DNA tracks the growing end of the filaments and the filaments form bipolar structures (Bharat *et al.*, 2015; Surovtsev, Campos and Jacobs-Wagner, 2016). The TubZ increases the number of roles of tubulin homologs in prokaryotic cells

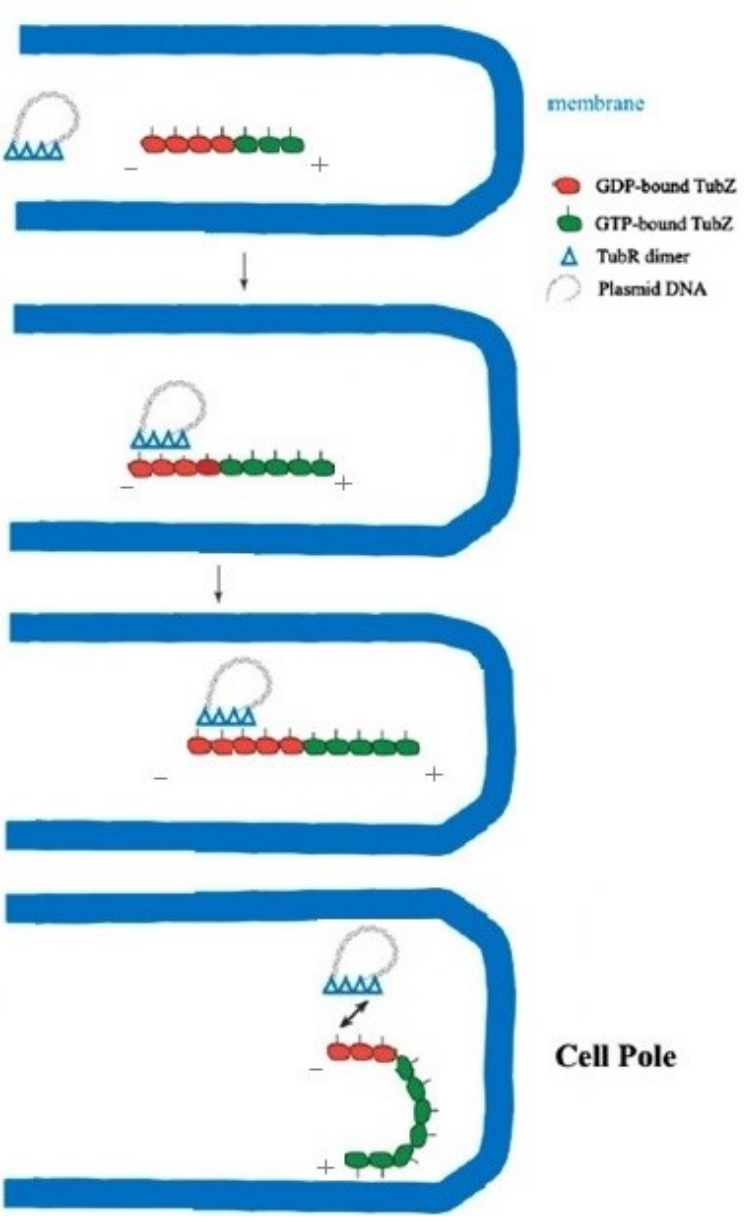

Figure 8: A model of the TubZRC plasmid partitioning system
The presumed model depicts the TubZ filament and its treadmilling through a cell. The TubRC complex trails the minus end of the filament. The TubRC complex then unbinds on the cell pole where the filament starts to curve (adapted from Ni *et al.*, 2010)

Interestingly, apart from TubZs in bacterial plasmids the TubZ-like sequences were also designated in Archaea and bacteriophages. The archaeal TubZ has not yet been researched thoroughly. The bacteriophage version of TubZ was renamed to PhuZ. The PhuZ filaments have sometimes different morphologies than the TubZ filaments, for example, a three-stranded filament from phage 201ϕ2-1. The function of the PhuZ filaments lies in clustering of the markedly big virus particles in the centre of the infected cell (Zehr *et al.*, 2014). More information is known about the PhuZ proteins but it is out of scope my thesis.

2.4. CetZ

First CetZ protein was initially named FtsZ3 and it is an archaeal tubulin homolog. It was described as a member of a distinct group of FtsZ/tubulin family and later renamed to CetZ for "cell-structure-related Euryarchaeota tubulin/FtsZ homologs". To date, it was recognized in genomes of various members of the Euryarchaea group. CetZ is often duplicated, one organism can contain up to 6 copies of slightly different CetZs in its genome. CetZ1 is the most conserved version. It is unclear whether the copies have different functions in cells (Vaughan *et al.*, 2004; Duggin *et al.*, 2015).

Some amino acid sequence features of CetZ as GTP binding and hydrolysis sequences are closer to tubulins whereas some other such as T1 loop sequence is closer to FtsZ. A crystal structure of CetZ1 and CetZ2 from *Haloferax volcanii* and CetZ from *Methanosaeta thermophila* revealed tubulin-like fold with C-terminal helices but without protruding N-terminal helices distinctive for FtsZ. CetZ2 with GTP-γS also formed straight protofilaments and even 2D sheets in vitro with lateral interactions similar to those in tubulin. The structure comparison is depicted in Figure 9 (Duggin *et al.*, 2015).

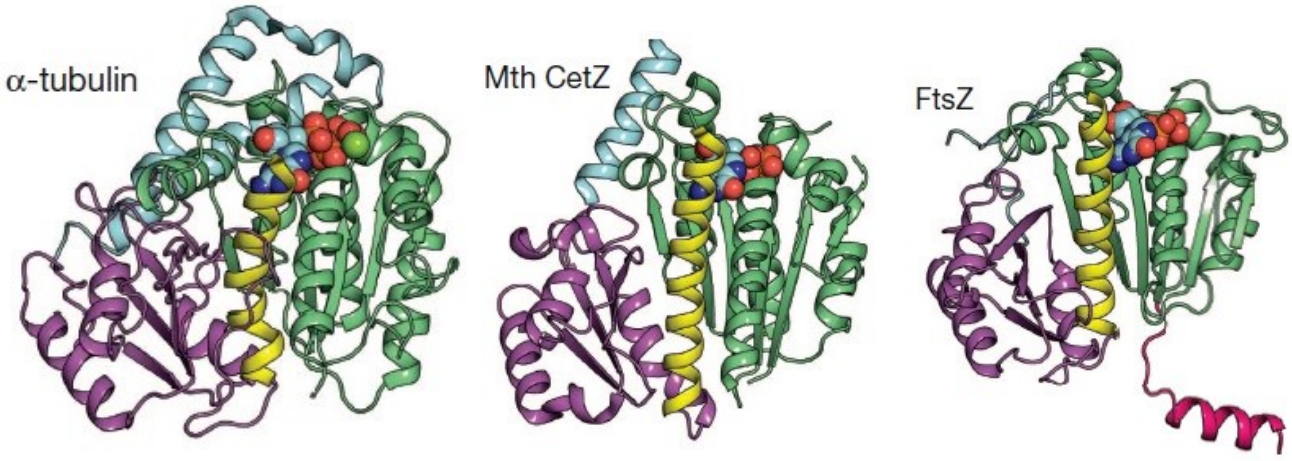

Figure 9: The crystal structure of CetZ in comparison to α-tubulin and FtsZ
Comparison of the different monomers. We can observe similar N domains in green, the FtsZ N-terminal H0 helix in red. The C-terminal domains are colored in purple with the H11 helix in cyan in tubulin and CetZ. The intermediate helices are in yellow. The H11 helix is a common feature of tubulin and CetZ and it is an important structure for subunit contact (adapted from Duggin *et al.*, 2015).

H. volcanii was used for *in vivo* experiments to gain information about the CetZ function. *H. volcanii* is a mesophilic and facultatively aerobic halophile. Its genome is completely sequenced and it is well cultivable in vitro. These features make *H. volcanii* a good model organism for studying Archaea (Hartman *et al.*, 2010).

CetZ always coexists with FtsZ in studied species genomes. *H. volcanii* knock-outs of CetZ and microbes with CetZ with unfunctional GTPase divided normally. On the other hand, depletion of FtsZ led to division defects. The FtsZ forms a ring at the mid-cell during the division of *H. volcanii.* Therefore, we can deduce that FtsZ has a cell division function in *H. volcanii,* not CetZ.

CetZ depletion leads to different malfunctions. Wild-type strain has two different morphotypes; irregular plate morphotype in the center of colonies and rod-shaped morphotype on the edges of colonies. The rod-shaped cells actively move from the center to the edges and further forming halos around colonies.

Colonies of CetZ-depleted strains contained a smaller number of rod-shaped cells on the edges and less rod-shaped cells in general. The colonies spread more slowly to their surroundings and formed smaller halos. Strains with overexpressed CetZ showed opposite behavior, the colonies were overall wider and contained more rod-shaped cells (Duggin *et al.*, 2015) GFP-tagged CetZ filaments were observed to move dynamically through the cell. The filaments moved along a longitudinal elongation axis of differentiating cells mainly near their cytoplasmic membrane. We can conclude that CetZ has a role in shape differentiation of *H. volcanii* and that the rod-shaped morphology is important for swimming motility. This movement could mean that CetZ is engaged in the cell remodelation to the rod-shaped cell (Duggin *et al.*, 2015). It is likely that CetZ has a similar function in other Archaea. This is the first example of prokaryotic tubulin homolog with function in cell shape control. However, the precise mechanism of CetZ action is yet to be determined. We do not have more information yet since the CetZ protein is a recently described protein. More experiments need to be performed so the precise mechanism of CetZ action can be revealed together with a deeper knowledge of its structure and dynamics.

2.5. Other tubulin superfamily sequences in Archaea

A high number of different FtsZ/tubulin family members can be found in Archaea. Apart from CetZ and FtsZ which were mentioned earlier, FtsZ-Like Group Homologs (FtsZL1) and artubulins sequences were discovered. More deeply-branching sequences are yet to be named and characterized (see Figures 10 and 11). However, these latter members have been studied only as DNA sequences so far. As a matter of fact, only few crystal structures of archaeal tubulin homologs are available; FtsZ1 from *Methanocaldococcus jannashii* (Oliva, Cordell and Löwe, 2004), CetZ1 and CetZ2 from *Haloferax volcanii* and CetZ from *Methanosaeta thermophila* (Duggin *et al.*, 2015). Archaea are largely difficult to cultivate and the vast majority of information about their life comes from studying their genomes. The genomes are often acquired through cultivation-independent methods and many features of the cells such as their morphology remain to be revealed. The same also applies to the tubulin homologs and therefore we know little about their localization or function in cells.

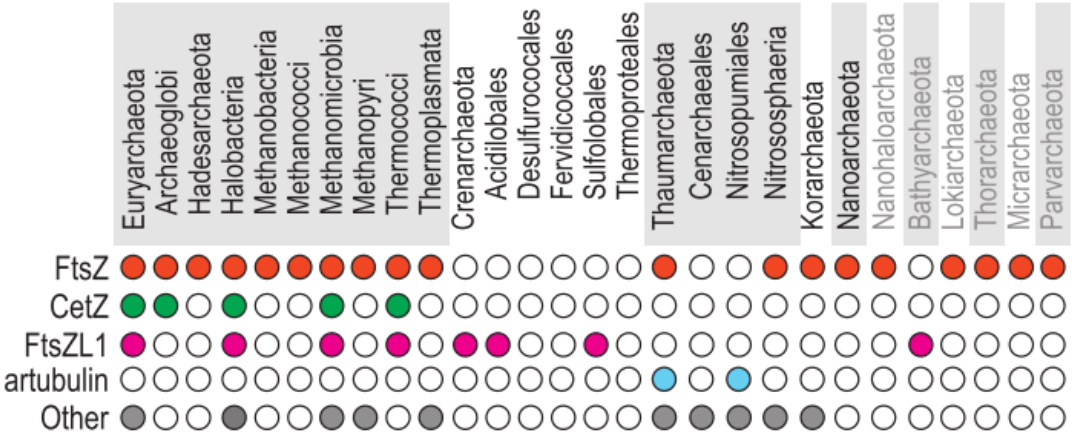

Figure 10: Presence of different members of tubulin family in Archaea
Filled circles mean the presence of the protein in at least one member of the archaeal taxa. Taxa in grey writing represent a candidate taxa or a group with only incomplete genome sequence available. The colors correspond to colors in Figure 11 (adapted from Aylett and Duggin, 2017).

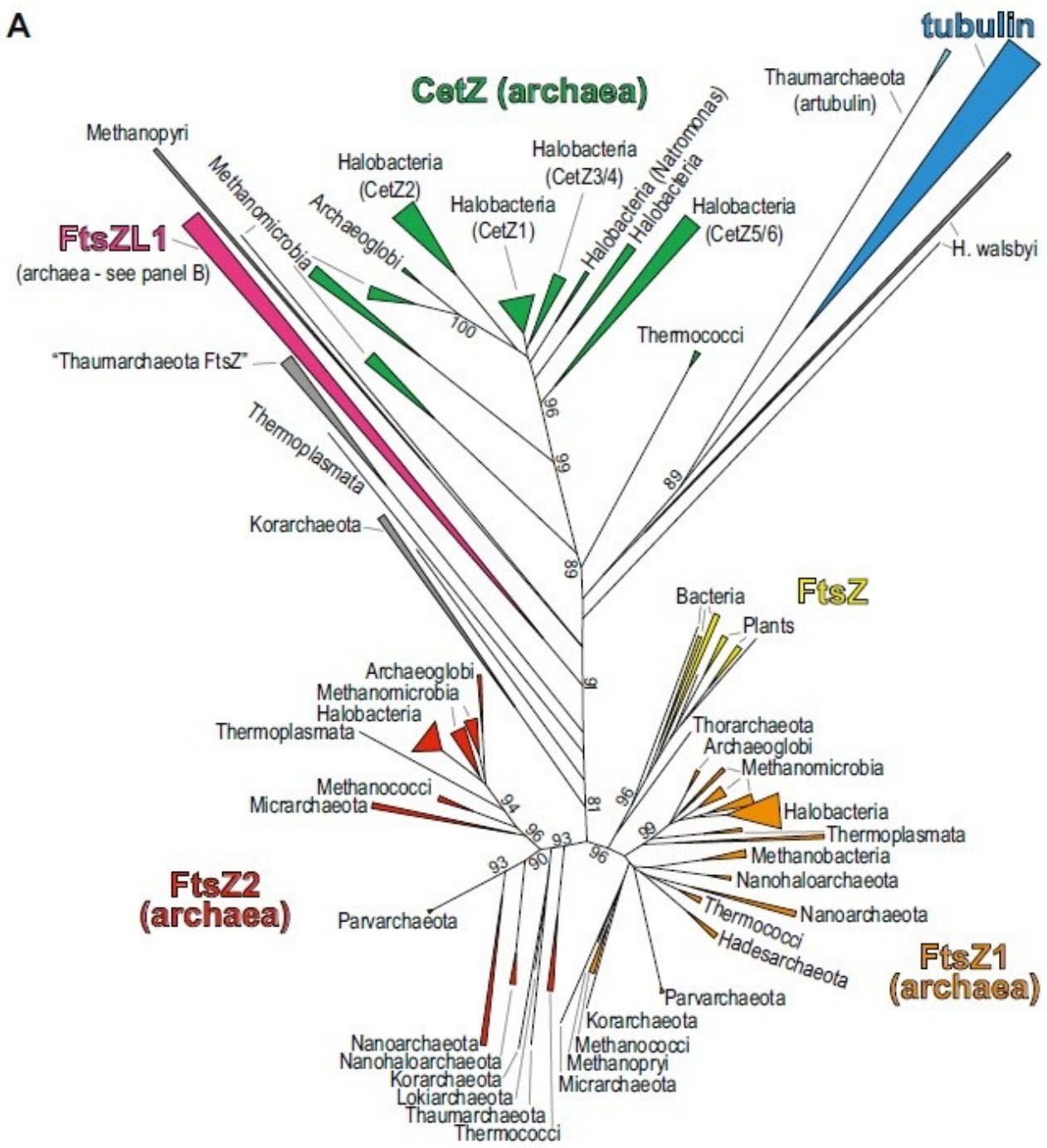

Figure 11: Phylogenetic tree of the tubulin superfamily in Archaea
The colored branches represent different tubulin superfamily members. FtsZ sequences from bacteria and chloroplasts are included, as well as eukaryotic tubulins. The grey branches represent uncharacterized sequences. Artubulins form a sister group to eukaryotic tubulins (adapted from Aylett and Duggin, 2017).

FtsZL1 group was identified on the basis of signature motifs of GTP-binding domain in some bacteria and archaea. FtsZL1 is not a group of FtsZ proteins rather a new group of tubulin homologs without a special name. Notably, it was the first discovered tubulin homolog in Crenarchaeota, a group which was thought not to possess any tubulin homolog. The Crenarchaeote FtsZL1 has a long N-terminal coiled-coil extension which possibly serves an unknown purpose. Also, the GTPase central region of the C-terminal domain seems to be bigger and unrelated to the other tubulin homologs, the GTPase-activation domain is missing. The authors of the discovery of these protein sequences suggest that the FtsZL1 proteins are unlikely to have a functional GTPase and to polymerize. They proposed that the proteins could have a function in membrane remodeling based on their genomic context of predicted peptidoglycan-binding proteins and GTPases (Makarova and Koonin, 2010). Further studies need to be realized to clarify the function of these features.

A very interesting group consists of so-called artubulins (archaeal tubulins) from the Thaumarchaeota. The alignment of the artubulins with eukaryotic tubulins from all seven families showed high similarity, the artubulins aligned with tubulins over a region of ~300 amino acids whereas they aligned only over a region of ~100 amino acids with FtsZ. The artubulins form a sister group to eukaryotic tubulins in the phylogenetic analysis. The artubulins genes are located next to subunits of the ESCRT-III complex (Yutin and Koonin, 2012). The ESCRT-III complex is a membrane remodeling system in eukaryotes (Raiborg and Stenmark, 2009). It has been described in some Archaea, mainly Crenarchaeota and Thaumarchaeota (Samson *et al.*, 2008; Spang *et al.*, 2015). The ESCRT-III complex was demonstrated to be essential for a cell division of these archaeons.
Furthermore, a thaumarchaeon with ESCRT-III complex which also encodes FtsZ does not use FtsZ as a cell division protein, it employs the ESCRT-III machinery (Samson *et al.*, 2008; Pelve *et al.*, 2011). It is possible that the artubulins are expressed together with the subunits of ESCRT-III complex in their neighborhood and they even may have a role in ESCRT-III division machinery.

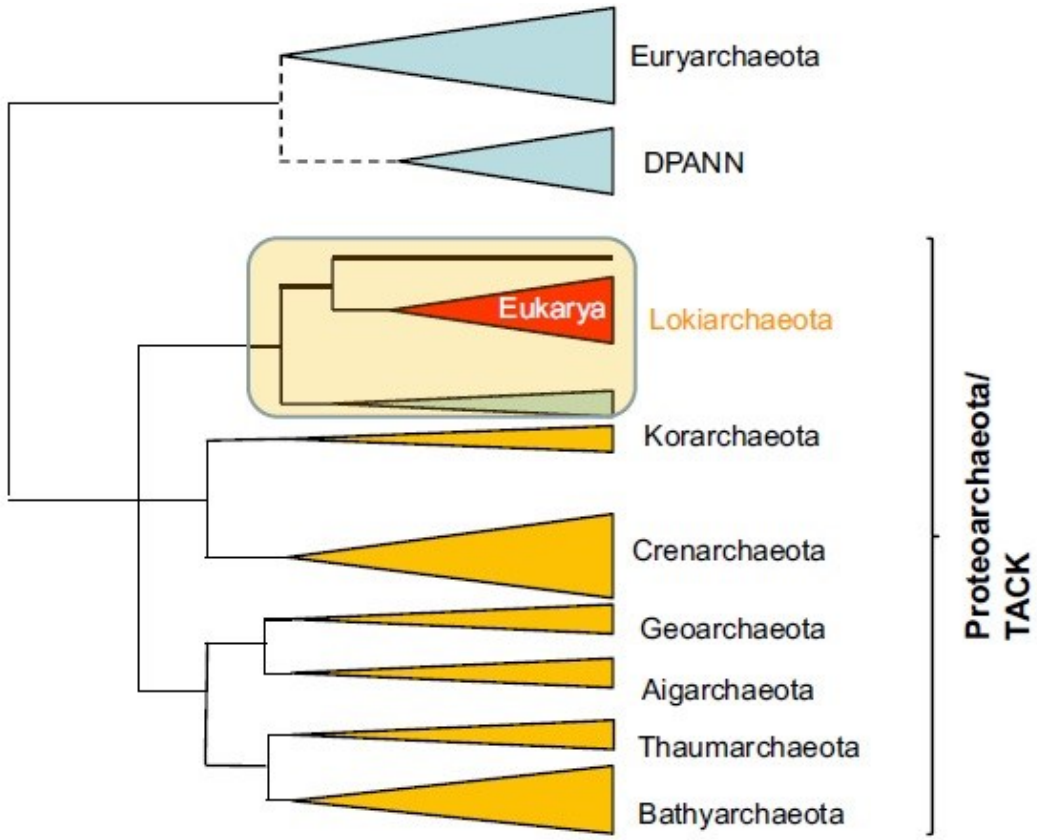

Figure 12: A schematic archaeal evolutionary tree
The tree represents major archaeal groups. The DPANN group is a proposed superphylum subsuming Nanoarchaota and other Archaea with small genomes. Eukaryota form a sister group Lokiarchaeota The size of the triangles represents the diversity of groups (adapted from Koonin, 2015)

Concerning these findings, the artubulins are likely ancestors of the eukaryotic tubulins. The artubulins are a sister group of all eukaryotic tubulins in contrast to bacterial tubulins ButbA/B which form a sister group to tubulins α and β (Yutin and Koonin, 2012). That would imply that the artubulins are descendants of an eukaryotic tubulin common ancestor. Concerning the wide prevalence of tubulin homologs in all domains of life, it seems that the tubulin superfamily universal ancestor was present in LUCA (last universal common ancestor) a presumed organism which was an ancestor to all present-day organisms (Weiss *et al.*, 2016). The eukaryotic tubulin ancestor was probably only one of many proteins of tubulin/FtsZ superfamily but it was conserved and passed onto the next eukaryotic generations. It seems that the eukaryotic tubulin ancestor might have come from within the Archaea. It is consistent with the contemporary opinion that eukaryotes originated as an inner branch of Archaea (see Figure 12) as a lot of eukaryotic features are scattered among different Archaea (Koonin, 2015) and that this group then underwent an endosymbiotic event, acquiring mitochondria (Spang *et al.*, 2015).

3. Conclusion

Prokaryotic tubulin homologs are a divergent group of proteins with various functions in cells. Even though their amino acid sequence homology with tubulin is mostly low they share similar crystal structure and dynamics with tubulin. The tubulin homologs widen our view of the cytoskeletal proteins as proteins with relatively uniform 3D structure can form a wide range of filaments. The various filaments presumably diverged in connection with their different functions in cells.

The eukaryotic tubulins are only one of many members of the tubulin/FtsZ superfamily and they probably originated from an archaeal ancestor.

While we have a quite deep understanding of the eukaryotic cytoskeleton, the prokaryotic cytoskeleton is still a relatively new subject. We are beginning to acquire a deeper understanding of some of the bacterial cytoskeletal proteins, their dynamics, and function in cells. However, there is a big gap in knowledge about archaeal cytoskeleton due to the aforementioned difficulties with research. This field is important to study because the archaeal cytoskeleton is probably the predecessor of the eukaryotic cytoskeleton. New methods in many fields will certainly help to enrich our knowledge about the archaeal cytoskeleton. Further research in both bacterial and archaeal tubulin homologs and other cytoskeletal proteins can help us understand the evolution of the eukaryotic cell and life in general and it surely is an interesting field of study.

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